

THE EXPERIMENTAL PRODUCTION OF POLYPLOIDY

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With plate 189

POLYPLOIDY has played an important rôle in the evolution of the angiosperms, and it is estimated that about half of the angiosperm species are polyploids. Two types of polyploids are known: autopolyploids, involving the reduplication of the same genom; and allopolyploids, involving the duplication of different genoms. The first type of polyploidy is not directly effective in speciation, although the polyploid differs from the diploid in certain morphological and physiological characters. Autopolyploidy does, however, serve to isolate the new form, since it is sterile with, and has a greater range than the diploid ancestor. In a recent survey of autopolyploidy Müntzing (1936) shows that such polyploids are generally more vigorous and hardier than the diploid forms and that the chromosome races are ecologically different. There is also a strong tendency for the polyploids to be perennials. Allopolyploidy is directly effective in producing new species, since such polyploids result from species hybridization, followed by chromosome doubling. The duplication of each of the parental genoms restores fertility, and the hybrid breeds true. Thus the allopolyploid is a constant species hybrid which has the characteristics of a true species, and in the case of generic crosses the allopolyploid may merit generic rank. Allopolyploids are known to occur in nature and in cultivation, and have been produced experimentally in several different families. The various types of allopolyploids have been described by Winge in 1932; and since that time a number of new cases have been discovered, so that we now have records of about fifty allopolyploids, of which eight are known to have originated in nature. It is significant that many of our most important crop plants are of known or supposed allopolyploid origin.

The general occurrence of polyploidy in the angiosperms has led to a detailed study of the causes of chromosome doubling in gametes and egg cells. In 1923 De Mol found that diploid pollen grains could be induced in *Hyacinthus* by subjecting the bulbs to abnormal environmental conditions. Belling (1925) found that low temperatures caused the production of diploid pollen grains in *Stizolobium*, *Uvularia*, and

Datura; and similar results were found in *Epilobium* and *Oenothera* by Michaelis (1928). The experimental work of Sakamura and Stow (1926) showed that high temperatures are also effective in producing diploid pollen grains. Shimotomai (1927) was able to produce giant pollen grains in *Liriope* and in *Scilla* by cold treatment. The effect of temperature treatment is produced by inhibiting the normal meiotic division so that no reduction of chromosomes occurs; but the factors involved are not well known.

The production of diploid gametes is undoubtedly an important factor in the formation of polyploid races or species in nature, even though sterile triploids would be formed more frequently than the relatively fertile tetraploids, especially in cross-pollinated species. The origin of polyploidy by the union of diploid gametes is dependent upon the frequent occurrence of chromosome doubling; but chromosome doubling in the fertilized egg, or at a later stage in somatic development, is directly effective in producing fertile polyploids. This mechanism is known to occur naturally, and has been used under experimental control. Randolph (1932) has been able to double the chromosome number in the fertilized egg cell of *Zea* by subjecting the plant to a temperature of 43°C. for about half an hour at the time the fertilized egg cell was presumably in the resting or early prophase stage. This technique has also been successful with wheat and rye (Dorsey 1936).

EXPERIMENTAL PRODUCTION OF POLYPLOID POLLEN GRAINS

During the past few years we have found occasional diploid pollen grains in plants of *Rhoeo discolor* grown in the greenhouse, where they were subject to moderate variations in temperature. Plants kept in the cold pit, at a temperature of about 5°C. for several days and then returned to the greenhouse, produced many diploid pollen grains. Last year we installed constant temperature chambers where the plants could be kept under control and studied more carefully.

We found that plants of *Rhoeo discolor* would produce some diploid pollen grains when subjected to either high or low temperatures for several days; but more consistent results were obtained when the plants were kept at a temperature of about 10°C. for two or three days and then kept at a temperature of 36°C. for one day. After treatment the plants were returned to the greenhouse and examined every few days.

Under normal conditions the chromosome pair at meiosis to form a ring or chain of 12 chromosomes. Adjacent chromosomes often pass to opposite poles so that each daughter cell receives six chromosomes.

These divide equationally to produce microspores with six chromosomes (Fig. 2). There is usually some irregularity in the chromosome distribution at meiosis, so that about 80 per cent of the pollen grains are sterile and undeveloped. The viable pollen grains contain an elongated dark-staining generative nucleus and a degenerate tube nucleus (Fig. 2A).

The first effect of the temperature treatment is the production of asynapsis. At metaphase the chromosomes are unpaired, and the twelve univalents lie on an equatorial plate (Fig. 1). The equational division of the univalent chromosomes produces a dyad with twelve chromosomes in each cell. In most cases these dyads develop independently to produce diploid microspores (Fig. 3) which develop into diploid pollen grains (3A). Since each microspore contains all twelve chromosomes, there is no deficiency of chromosomes or chromosome segments, and the microspores produce normal pollen grains. There is very little sterility of diploid pollen grains, and certain anthers show almost perfect pollen development as contrasted with about 80 per cent sterility in the normal diploid.

The temperature variation also produces tetraploid microspores. Meiosis is suppressed, and the twelve univalents divide, but there is no nuclear division. The chromosomes pass into the resting stage, and the entire microsporocyte develops as the microspore. There are twenty-four chromosomes in the microspore which divide to form the two nuclei of the pollen grain (Fig. 4). The differentiation of the generative and tube nucleus is seldom complete, although a few giant pollen grains are produced which appear to be normal (Fig. 4A). These tetraploid pollen grains are not produced regularly, and a single anther never contains only this class of pollen grains. They are found in small numbers associated with haploid pollen grains.

In rare cases the univalent chromosomes divide, and the daughter nuclei are formed, but no cell wall is formed to produce a dyad. The microsporocyte functions as a microspore with two nuclei, each with twelve chromosomes. In microspore development each nucleus divides (Fig. 5), and a pollen grain is produced which has two generative and two tube nuclei (Fig. 5A).

Diploid pollen grains have also been produced in species of *Tradescantia*, but only when the plants have been subjected to low and high temperatures. Cold or heat treatment alone is rarely effective in producing polyploid gametes in this genus. The first indication of reaction to temperature treatment is partial asynapsis; further reaction causes complete asynapsis with irregular division of chromosomes; and with

extreme treatment, the univalents divide equationally to form diploid pollen grains.

The effect of temperature treatment appears to be caused by disturbing the synchronization of nuclear and cytoplasmic activities. This effect is especially clear in the development and differentiation of the generative and tube nucleus in microspore development in *Tradescantia* (Sax 1935). In the microsporocyte the temperature change prevents chromosome pairing. The development of the chromosomes is accelerated in relation to other nuclear and cytoplasmic activities so that the univalents are ready to divide at the first instead of the second meiotic division. They may divide before nuclear division is possible so that the chromosome number is doubled. This is the mechanism involved in the production of tetraploid pollen grains of *Rhoeo* and the tetraploid egg cells of *Zea* and *Triticum*.

THE ORIGIN OF POLYPLOIDY IN NATURE

The occurrence of polyploidy in nature has been attributed to extreme temperatures. The tetraploids may have a more northern range than the diploids (Tischler 1935, Anderson and Sax 1936), or a more alpine distribution (Manton 1934), or may show a greater distribution along the seacoast (Tischler 1935). In such habitats a species would be subjected to greater temperature variations. The extreme temperature changes may cause polyploidy as the diploid races extend into unfavorable territory, but it is also possible that the polyploids have originated before the extension of the range, and because they are hardier, they extend their range into more extreme environments.

There are a number of cases where the forms in the northern areas or in extreme environments are more frequently diploid than the related varieties or species in more favorable environments (Böcher, 1936; Rohweder, 1936). One might expect that any extreme change in habitat might induce polyploidy, and that polyploidy would be expected at the periphery of the range, either northern or southern, or at low or high elevations. There is some evidence that different genera vary greatly in response to temperature changes. In many cases there is a definite cycle in gamete development which may serve as an adaptation to extreme diurnal temperatures. In the cereals, for example, the meiotic divisions tend to occur between 6 A. M. and 8 A. M., and it is possible that the more critical prophase stages are synchronized so that they are not often subjected to great variations in temperature.

The experimental evidence obtained with *Rhoeo* and *Tradescantia* indicates that polyploidy may be induced by rather moderate tempera-

ture changes in certain genera, but that rather extreme and sudden changes are more effective. Neither constant heat nor constant cold seems to be effective. Since extreme variations in temperature are most likely to cause polyploidy, one might expect polyploidy in nature to occur in regions of high altitude, in semi-arid regions, or along the sea-coast, where the changes in temperature are often rapid and extreme. The polyploid economic plants appear to have originated in such regions. Of the seven centers of distribution described by Vavilov (1932), five are regions of high elevation. Most of the economic plants of polyploid origin—including apples, pears, wheat, oats, potatoes, tobacco, and upland cotton—have originated in the mountains or foothills in the subtropics or tropics.

The increased vigor and hardiness of the polyploids are known to be associated with an increase in range of distribution in certain genera. We should expect that polyploids might be more frequent than diploids at the periphery of the range. Many species of plants in North America originated in Asia. A comparison of Asiatic and American species grown in the Arnold Arboretum shows that polyploidy is more frequent in the American species of *Malus*, *Rosa*, *Acer*, *Staphylea*, and *Ulmus*, while the reverse is true for *Lonicera* and *Fraxinus*. Many genera, such as *Rhododendron*, are represented entirely, or almost completely, by diploid species in both the old and new world. The data are hardly adequate for a critical survey, but the significance of polyploidy undoubtedly varies in different genera.

EXPERIMENTAL POLYPLOIDY

Work on experimental polyploidy is being conducted at a number of research laboratories. Belling (1925) has called attention to the value of triploids in ornamental plants where seed production is not desired. The use of diploid gametes, formed without a reduction division, should be of value in breeding heterozygous horticultural varieties. In the apple, for example, the varieties are so heterozygous that breeding new varieties is largely a matter of chance; but the union of diploid gametes should combine all the characters of the parental trees.

In the production of auto- and allopolyploids Randolph's method of doubling the chromosome number in the fertilized egg seems to be the most practicable. Our experimental work indicates, however, that it is advisable to precondition the plant in a cold chamber before subjecting the fertilized egg to high temperatures. I have been told that the heat treatment alone is not effective in doubling the chromosomes in *Zea* in the southern states. The failure of chromosome doubling in southern

regions presumably is caused by the relatively high temperatures of the normal environment, so that there is insufficient range of temperature to cause chromosome doubling. Under such conditions the cold treatment is necessary, and probably most plants would show a better response when subjected to an extreme temperature range.

Either the transition from cold to heat or from heat to cold seems to be effective in causing polyploidy. In experimental work the cell activities can be timed somewhat better if the plant is first subjected to cold treatment.

SUMMARY

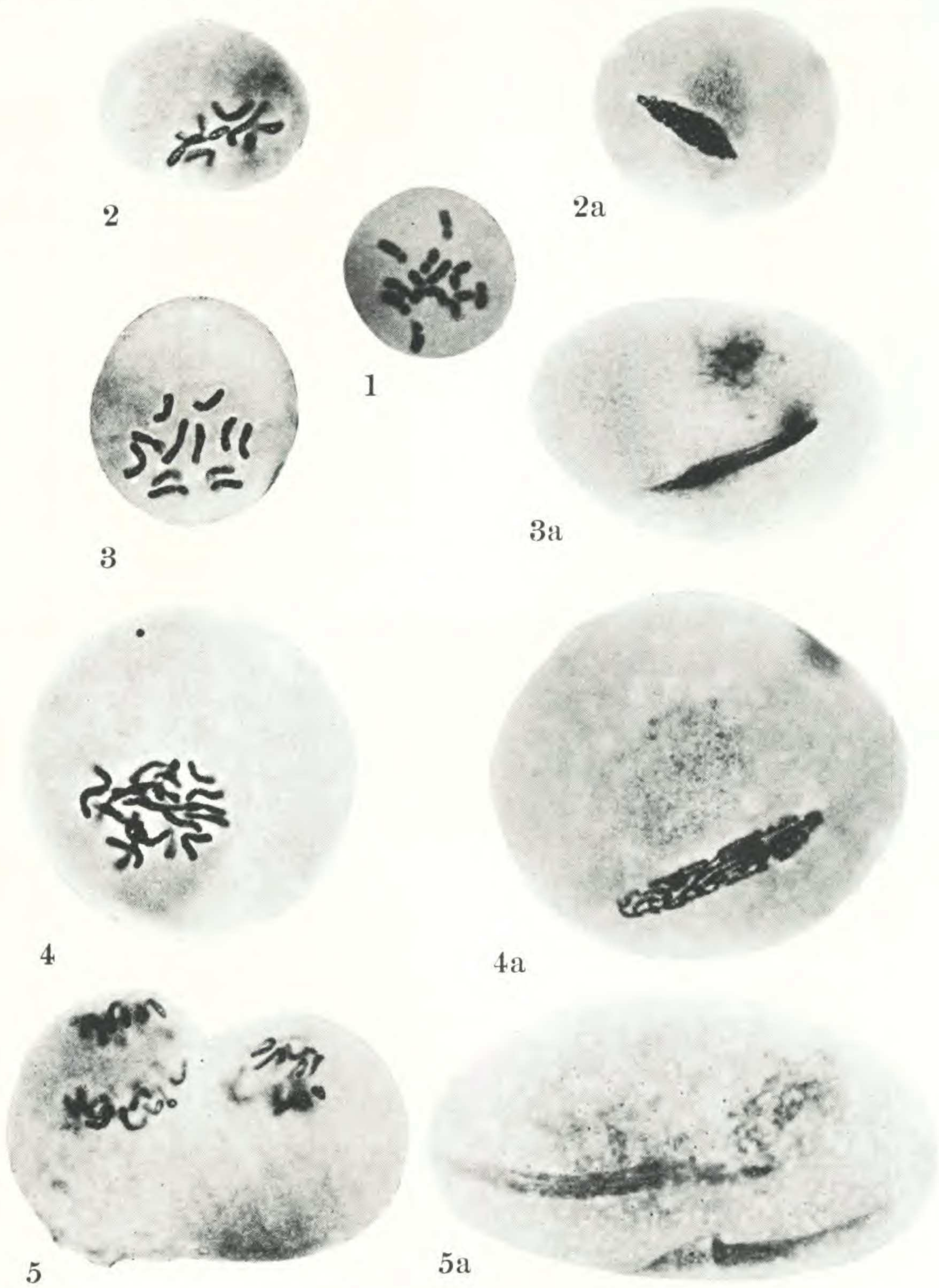
Plants of *Rhoeo discolor* subjected to sudden temperature changes produced many diploid pollen grains, and a few tetraploid pollen grains. Chromosome doubling is caused by the suppression of meiosis and an equational division of the chromosomes to produce dyads which develop into diploid pollen grains. Occasionally the chromosomes divide without nuclear division to produce a monad which develops into a tetraploid pollen grain. Double diploid pollen grains may be produced which have two generative and two tube nuclei. Chromosome doubling appears to be caused by disturbing the synchronization of nuclear and cytoplasmic activities.

The effect of temperature variations in causing polyploidy in nature is discussed in relation to plant distribution and the centers of origin of cultivated plants. In the experimental production of polyploidy extreme temperature changes are more likely to be successful.

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LITERATURE CITED

- ANDERSON, E., and KARL SAX (1936). A cytological monograph of the American species of *Tradescantia*. (Bot. Gaz. **97**: 433-476.)
- BELLING, J. (1925). Production of triploid and tetraploid plants. (Jour. Hered. **16**: 463-464.)
- BÖCHER, T. W. (1936). Cytological studies on *Campanula rotundifolia*. (Hereditas, **22**: 269-277.)
- DORSEY, E. (1936). Induced polyploidy in wheat and rye. (Jour. Hered. **27**: 155-160.)
- MANTON, I. (1934). The problem of *Biscutella laevigata*. (Zeitschr. Indukt. Abst. Vererb. **67**: 41-57.)
- MICHAELIS, P. (1928). Ueber die experimentelle Erzeugung heteroploider Pflanzen bei *Epilobium* und *Oenothera*. (Biol. Zentralbl. **48**: 370-374.)
- MOL, W. E. DE (1923). Duplication of generative nuclei by means of physiological stimuli and its significance. (Genetica, **5**: 225-272.)



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